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Isolation, Characterization and Identification of Lactic Acid Bacteria from Ready to Consume *Shamita*: Ethiopian Traditional Fermented Beverage

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ABSTRACT
The study was conducted in sub-city of Addis Ababa to isolate, characterize and identify Lactic acid bacteria (LAB) from ready to consume S*hamita*. A total of forty five samples of ready to consume shamita were collected from randomly selected household brewers from three purposively selected sub-cities namely (Gulele, Arada, Yeka). For isolation of lactic acid bacteria, samples were serially diluted up to 10^{-6} and plated on MRS agar at 32^{o}C for 48hours in anaerobic condition. Well-isolated colonies with typical characteristics were picked from each plate and were further sub cultured until pure isolates were obtained. Purely isolated colony was identified by cell morphology, cell arrangement, gram-staining, catalase test and acid production from glucose. The data were analyzed using Statistical Package for Social Sciences (SPSS) (2011) software (ver.20). A total of 35 lactic acid bacteria isolates belonging to five genera Leuconostoc (31.43%), Pediococcus (8.57%), Lactobacillus (22.86%), Streptococcus (17.14%), Lactococcus (20%), were characterized and identified. Streptococcus, Leuconostoc, Pediococcus and Lactococcus were cocci shaped. In the present study the dominant genera of lactic acid bacteria identified from shamita samples were leuconostoc (31.43%). Therefore, traditional fermented beverages like *Shamita* can be a good source of lactic acid bacteria.

Key words: Ethiopia, Identification, Isolation, Lactic acid bacteria, Shamita, Traditional fermented beverages.

INTRODUCTION

Fermentation is one of the effective and the most economical methods of producing and preserving foods and beverages acceptable to man. Biologically fermentation can enrich food substrates with protein, essential amino acids, essential fatty acids and vitamins; enhance the diet through a diversity of flavors, aromas and textures; and decrease cooking time and fuel requirements [1]. Microbiologically, fermentation of traditional fermented products relies on the microorganisms particularly (LAB) present in the substrates, fermenting vats and equipment's. Lactic acid bacteria are the most frequently encountered groups in almost all fermented products. Fermented products exhibit great stability with the ability to dominate over the wide variety of microbes which will inevitable present in grains where the food is prepared by traditional methods without the benefit of modern cleaning and sterilization agents. Fermented foods and beverages constitute a major portion of people's diets in Africa [2].

Ethiopia is one of the countries where varieties of traditional fermented foods and beverages are produced and consumed. The major indigenous fermented foods which are produced in Ethiopia are *Borde, Shamita, Tella, Tej, Araki, Korefe, Keriboand and Duka* [3]. *Borde* and *Shamita* are important traditional fermented beverages in Ethiopia. As indicated by [4-5] *Shamita* is very popular traditional fermented beverage mainly prepared in central and southern Ethiopia, and mainly consumed as meal replacement by low–income groups. *Shamita* is a widely consumed low alcohol beverage with a thick consistency and is consumed as meal replacement by most people who cannot afford a reasonable meal in Ethiopia. Malt is not commonly used in *shamita* fermentation, although local *shamita* brewers in Addis Ababa use it frequently, and starch is the only principal fermentable carbohydrate. It is assumed that the microorganisms responsible for the fermentation come mostly from back-slopping using a small amount of *shamita* from a previous fermentation /starter culture /as well as from ingredients and equipment's used for preparation. Laboratory prepared *shamita* had comparable microbial counts with samples obtained from local *shamita* brewers in Addis Ababa [6].

Previous studies indicated that the fermentation processes of *shamita* mainly involved lactic acid bacteria [4, 6-8]. The microbial sources of both *borde* and *shamita* included ingredients;

fermentation utensils, fermenting pot, and back slopping [6, 9]. As a fermented product shamita is highly probable to harbor a group of lactic acid bacteria.

Lactic acid bacteria (LAB) are a group of Gram-positive, non-sporulating, anaerobic or facultative aerobic cocci or rods, which produce lactic acid as one of the main fermentation products of the metabolism of carbohydrates [10]. LABs are a group of morphologically, metabolically and physiologically similar bacteria and they are relatively closely related phylogenetically. According to [11-12] fermenting organisms include LAB such as, Leuconostoc, Streptococcus, Lactobacillus, Enterococcus, Aerococcus and Pediococcus spp. Lactic acid bacteria are industrially important organisms recognized for their fermentative ability as well as their health and nutritional benefits.

Lactic acid bacteria (LAB) have been used for the fermentation of food and feed products since ancient days and today their major applications are still in the food and feed industry as starter cultures [13-15]. Fermented foods and beverages are a good source of lactic acid bacteria. Thus, the objective of this study was to isolate, characterize and identify lactic acid bacteria from ready to consume *shamita* from selected sub-cities of Addis Ababa.

MATERIALS AND METHODS

SAMPLE COLLECTION

Three sub-cities namely (Gulele, Arada, Yeka) were purposively selected for the study. Sub cities were selected based on their potential of *shamita* preparation. Sample collection was done from April to May 2013. A total of forty five household brewers fifty from each sub city were randomly selected. A total of 45 samples (250ml) of ready to consume *shamita* were collected from selected household brewers using sterilized flasks and brought to laboratory for microbiological analysis. Samples were kept in a refrigerator (around 4°C) till the analysis begins.

ISOLATION OF LACTIC ACID BACTERIA

For isolation of lactic acid bacteria, samples of *shamita*, (25ml) were homogenized with 225ml sterilized buffered peptone water for about 1-3 minutes aseptically. From the homogenate mixture 1ml of suspension was transferred in to a test tube containing 9ml of buffered peptone water and test tubes were mixed using a vortex and dilution was made up to 10^{-6}. A volume of 0.1 ml of appropriate dilutions of *shamita* was plated on MRS (OXOID) agar plates. Then the plates were incubated for 48hours in anaerobic jar at 32°C.

CHARACTERIZATION AND IDENTIFICATION OF LACTIC ACID BACTERIA

Fifty five individual isolates/colonies from MRS agar plates were randomly-picked, representatives from all morphologically distinct colonies and were sub-cultured and purified 3 times on the appropriate MRS agar medium. Identification of LAB was done based on morphology, physiology and biochemical characteristic [16]. Forty three pure bacterial isolates were further tested for cell morphology, motility, gram reaction, catalase production, acid production from glucose and growth at 10^0C, 15 ^{0}C, 45 ^{0}C and 37^0C as a control according to the methods described by [8]. Cell morphology of cocci or rod shaped, non-motile, gram-positive, catalase-negative, isolates with characteristic cell arrangements were considered as lactic acid bacteria isolates.

RESULTS

A total of 35 lactic acid bacteria were isolated from ready to consume *shamita* collected from three sub-cities of Addis Ababa. After morphological and gram test characterization 27 of them were determined as representatives of the lactic acid cocci, and the rest 8 isolates were rod shaped. Eventually isolates were grouped as cocci and rods based on cell shape, and cell arrangement observed under light microscope.

All isolates were initially subjected to preliminary tests including gram reaction, motility test, catalase reaction, cell morphology and cell arrangement [17]. With that, any yeast and gram negative bacteria are excluded from the next step of identification. Only gram positive, rod and cocci shape bacteria were chosen as they represent the LAB characteristics. Initially 43 colonies were selected and subjected to cell morphology and gram test. However, only 35 isolates were shown gram positive and cocci or rod shapes which is the characteristics of LAB.

Further screening procedure were performed for the isolates by inoculating cell in modified MRS medium for motility test, and results showed that all isolates were non-motile. Genus differentiation of lactic acid bacteria are usually based on Gram staining, catalase test, and determination of carbohydrate utilization. All the 35 isolates were catalase negative. Morphological, physiological and biochemical characteristic of identified LAB are shown in (Table 1).

Morphologically cells from 35 isolates, 27 of the isolates were cocci in shape out of them 11 arranged in pair/chain 3 of the isolates were tetrad/chain, 6 were spherical or ovoid and the

remaining 7 isolates were arranged ovoid. The cultural characteristics of the above isolates on MRS agar were circular, convex and cream in color. The cells of remaining 8 isolates were rod shaped in cell type and arranged in pair or chains. Their colony morphology on MRS agar was circular, convex non-pigmented and entire margin.

From 8 rod shaped isolates 5 isolates were positive for growth at 10°C, 15°C, 37°C and 45°C. However 3 isolates were slow growth at 45°C. From 27 cocci shaped isolates 20 isolates were positive growth at 10°C, 15°C, 37°C and 45°C followed by 4 isolates were slow in growth and the remaining 3 isolates were undetermined growth at 45°C. Generally, the majority of the isolates were best grown at 37°C. Acid production from glucose was determined by inoculating the isolates in MRS broth media and incubated at 37°C.

Table1. Morphological, physiological and biochemical properties of isolated genus

Isolated genus	Cell shape	Cell arrangement	Gram test	Catalase test	Acid production from glucose
Leuconostoc	cocci	Pair or short chain	+ve	-ve	+/-
pediococcus	cocci	Tetrads in pair or short chain	+ve	-ve	+/-
Lactobacillus	rod	Tetrads in pair or short chain	+ve	-ve	+/-
Streptococcus	cocci	Spherical and ovoid	+ve	-ve	+
Lactococcus	cocci	Spherical	+ve	-ve	+/-

+ = Positive reaction; – = Negative reaction; +/- =varies between species

All isolates are positive for acid production from fermentation of glucose. However in some genus acid production was varies between species. This means some of the isolates were slow and others were fast or high in acid production. The genus of LAB isolates was identified by comparing to the LAB identification table [18-19]. In addition, the determination of the isolates in to genus level was performed according to their morphological, cultural, physiological and biochemical characteristics by the procedures described in the Bergey's Manual [20]. Percentages of isolated lactic acid bacteria genus are shown in (Figure1). The LAB isolates were classified into the genera Streptococcus, Leuconostoc, Pedio- coccus and Lactobacillus based on their morphology and biochemical characters [21]. With this, the isolated LAB was identified as the genus Lactococcus (20%), Pediococcus (8.57%), Lactobacillus (22.86%), Streptococcus (17.14%), Leuconostoc (31.43%). From all the isolated lactic acid bacteria the genus leuconostoc is the dominant.

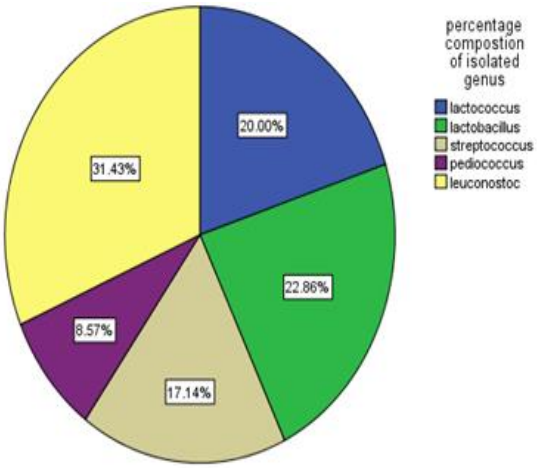

Fig.1Percentage of isolated LAB genus

DISCUSSIONS

Lactic acid bacteria (LAB) belong to a group of gram positive bacteria that produce lactic acid as their main fermentation product into the culture medium and generally recognized as safe [22]. Traditional fermentation of beverages like *shamita* by the process of lactic acid fermentation has numerous advantages beyond those of preservation. The growth of LAB in fermented beverage enhances bioavailability of nutrients. LABs are bacteria in rod or coccus shapes, with negative catalase, not motile, homo fermentative or hetero fermentative and growing in low acid condition [3]. Lactic acid bacteria (LAB) are one of the microorganisms that dominate fermented food [23-24]. Today, LAB is of essential importance for their role in most industries of fermented foods as starter cultures.

In the present study 35 isolates of lactic acid bacteria belonging to five genera from ready to consume *shamita* were identified. All the isolates were non-motile, catalase negative, gram positive and cooi /rod shaped. LAB species isolated from goat milk were rod and cocci shaped either chained or clustered, and characterized by positive Gram, negative catalase, microaerophylic, resistant to acid, not producing spore, and producing lactic acid as fermentation product [25]. In addition, [26] indicated that LAB were bacteria with morphological, physiological and metabolical characteristics belong to positive Gram group, with negative catalase, coccus or round shaped, without

spore, not motile, without respiration, but can grow on aerobic or microaerotolerant conditions, and can produce lactate acid as main metabolic product from carbohydrate fermentation.

Lactic acid bacteria produce lactic acid as their main end product of carbohydrate fermentation. As a result, lactic acid not only keeps foods and beverages in the state of perfect preservation but can also promote the growth of healthy microbiota throughout the intestine beside of reducing the pathogens. LAB species are very important in food industry as beneficial organisms. One of the most important contributions of these microorganisms is the extended shelf life of the fermented products. Lactic Acid Bacteria are generally associated with habitats rich in nutrients, such as various food products (milk, meat, vegetables), but some are also members of the flora of the mouth, intestine and vagina of mammals.

Naturally, LABs grow to large numbers during the fermentation of the products [6, 8] this indicates that isolation of LAB is also possible from traditional fermented foods and beverages. The result of the current study, were comparable with reported by [27] lactobacillus; Pediococcus, Streptococcus and Leuconstoc were isolated from borde and *shamita.* In addition, [28] indicated that L. fermentum, Pediococcus spp, Leuconostoc spp, Enterococcus spp, C. humilis and S. exiguus have been isolated from sourdough. The present study had great success in isolating lactic acid bacteria from ready to consume *shamita* samples by agar plate culture.

In all sampled beverage examined, LAB are the dominant microorganisms, and therefore, lactic acid fermentation is considered as the major contributor to the beneficial characteristics observed in fermented beverages. This is due to the fact that Lactic acid bacteria are highly involved in fermentation process and fermented products. Fermenting organisms include LAB such as, *Leuconostoc, Streptococcus, Lactobacillus, Enterococcus, Aerococcus* and *Pediococcus* spp. [11-12, 28-29].

CONCLUSIONS

In the present study, a total of 35 isolates of LAB belongs to the genus lactobacillus, lactococcus, lecunostoc, streptococcus and pediococcus were identified from ready to consume *shamita*. From this study, we can conclude that fermented beverages particularly *shamita* is a rich source of LAB. Traditionally fermented *shamita* contained both homofermentative and hetrofermentative lactic acid bacteria. Therefore, consuming traditionally fermented foods and beverages like *shamita* are important as they contained probiotic lactic acid bacteria. Further studies will be done on the characterization, identification in to species level and production of important biomolecules by identified species.

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REFERENCES

[1] Steinkraus K. H. (1996) Introduction to Indigenous Fermented Foods. In: Handbook of Indigenous Fermented Foods (K. H. Steinkraus, ed.). Marcel Dekker, New York, 1-5.

[2] Oyewole B. (1997) Lactic fermented Foods in Africa and their benefits. Food Control. 8: 289-297.

[3] Holzapfel E.H., Haberer P., Geisen R., Björkroth J., Schillinger U. (2001) Taxonomy and important features of probiotic microorganisms in food and nutrition. American Journal of Clinical Nutrition 73(2): 365S-373S.

[4] Ashenafi M., Mehari T. (1995)Some microbiological and nutritional properties of “borde” and “shamita”, traditional Ethiopian fermented beverages. Ethiopian Journal of Health Development 9: 105 - 110.

[5] Ashenafi M. (2002) The microbiology of Ethiopian foodsand beverages: a review. SINET: Ethiopian Journal of Science 25 (1): 97 – 140.

[6] Bacha K., Mehari T., Ashenafi M. (1999) Microbiology of the fermentation of ‘Shamita’, a traditional Ethiopian fermented beverage. SINET: Ethiopian Journal of Science 22: 113 – 126.

[7] Abegaz K., Langsrud T., Beyene F., Narvhus J.A. (2004) The effect of technological modification on the fermentation of borde, an Ethiopian traditional fermented cereal beverage. Journal of Food Technology in Africa 9: 3-12.

[8] Abegaz K. (2007) Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of borde, an Ethiopian cereal beverage. African Journal of Biotechnology 6: 1469- 1478.

[9] Abegaz K., Beyene F., Langsrud L., NarvhusJ.A. (2002) Indigenous processing methods and raw materials of borde, an Ethiopian traditional fermented beverage. Journal of Food Technology in Africa 7: 59- 64.

[10] Hayek S.A., Ibrahim S.A. (2013) Current Limitations and Challenges with Lactic Acid Bacteria: A Review. Food and Nutrition Sciences, 4, 73-87.

[11] Blandino A., Al-Aseeri M.E., Pandiella S.S., Cantero D., Webb C. (2003) Cereal-based fermented foods and beverages. Food Research International.36(6):527-543.

[12] Antony U., Chandra T.S. (1997) Microbial population and biochemical changes in fermenting finger millet (Eleusinecoracana). World Journal of Microbiology and Biotechnology.13(5):533-537.

[13] Desmons S., Krhouz H., Evrard P., Thonart P. (1998) Improvement of lactic acid cell production. Applied Biochem. Biotechnol. 70-72(1): 513-26.

[14] Van Casteren., W.H.M., Dijkema C., Schols H.A., Beldman G., Voragen A.G.J. (1998)Characterisation and modification of the exopolysaccharide produced by Lactococcuslactissubspcremoris B40. Carbohydr. Polym. 37(2): 123-30.

[15] Boonmee M., Leksawasdi N., Bridge W., Rogers P.L. (2003) Batch and continuous culture of Lactococcus lactic NZ133: experimental data and model development. Biochem. Eng. J. 14(2): 127-35.

[16] Harrigan W.F.,McCance M.E. (1976) Laboratory methods in food and dairy microbiology, Acad. Press, 1st Ed., London, 25-29

[17] Kimaryo V., Massawe G., Olasupo N., Holzapfel W. (2000) The use of a starter culture in the fermentation of cassava for the production of "Kivunde", a traditional Tanzanian food product. Inter. J. Food Microbiol. 56: 179-190.

[18] Axelsson L. T. (1993) Lactic acid bacteria classification and physiology. In: S. Salminen and A. V. Wright. (eds.). Lactic acid Bacteria. New York: Marcel Dekker., 1-66.

[19] Roissart H., Luquet F.M. (1994) Bactérieslactiques:Aspects fondamentaux et technologiques (tome1). Paris, Lorica, 605.

[20] Krieg N. (1984) Bergey's Manual of Systematic Bacteriology. (Ed), vol. 1, 2, Baltirnore: Williams and Wilkins.

[21] Sharpe M. E., Fryer T. F., Smith D. G. (1979) Identification of Lactic Acid Bacteria. In: Identification Methods for Microbiologists, E. M. Gibbs, F. A. Skinner (Eds), London: Academic Press, 233-259.

[22] Konings W.N., Kok J., Kuipers O.P., Poolman B. (2000) Lactic acid bacteria: the bugs of the new millennium. Current Opinion in Microbiology 3 (3): 276-282.

[23] Guasch J. M., Andrés L. C., Jáuregui O., Lamuela R. R .(2005) First evidence of white wine in ancient Egypt from Tutankhamun's tomb. J. Archaeol. Sci. 33: 1075-1080.

[24] Robert S. (2008) Ecology of fermented foods. Human Ecology Rev. 15: 25-31.

[25] Tserovska L., Steno S.,Yordaufa T.(2004) Identification of Lactic Acid Bacteria Isolated from Katyk Goat milk and Cheese. J Cult collection. Bank IndustriMic Cell Cult 13: 48-52.

[26] Tadese G., Ephrim E.,Ashenafi M. (2005a) Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some food-borne pathogens and Ethiop. J. Biol. Sci., 5(2): 189-245.

[27] Vuysta L., Vancanneyt M. (2007) Biodiversity and identification of sourdough lactic acid bacteria. J. Food Microbiol. 24: 120-127.

[28] Hirayama K., Rafter J. (1999) The role of lactic acid bacteria in colon cancer prevention: mechanistic considerations. Antonie vanLeeuwenhoek, 76, (1):391-394.

[29] Holzapfel W. (2002) Use of starter cultures in fermentation on a household scale. Food Control. 8(5-6):241-258.